the amount of $890.00 in payment of the required fee therefor. Any deficiency or overpayment should be charged or credited to Deposit Account No. 50-0320.

A Notice of Appeal, in triplicate, together with the required fee therefor, is being filed concurrently herewith.

The instant invention relates to improving plant growth by the expression of at least one bacterial asparagine synthetase in the choloroplast and/or plastid of cells of the plant. The invention is also directed to methods for so improving plant growth by, inter alia, introducing a nucleic acid molecule encoding the bacterial asparagine synthetase into the plant genome (e.g., into plant cells and culturing and/or regenerating the cells into the plants) wherein the nucleic acid molecule is operably linked to a nucleic acid molecule comprising regulatory sequences for expression and for import of the bacterial asparagine synthetase into the chloroplast and/or plastid; and, to plants, having such improved growth.

Claims 9, 11 and 13 have been objected to because of certain informalities. These claims have been amended herein to address each of these objections and thereby overcome same.

Claims 9 and 11 have been rejected and claim 12 remains rejected under 35 U.S.C. §112, second paragraph, as being indefinite, for reasons of record as set forth in the previous Office Action.

Specifically, claim 9 is said to be indefinite in its recitation of "chloroplastic GS-antisense RNA" in step C. Claim 11 has been criticized with respect to the recitation of the language "the imported asparagine synthetase"; claim 12 has been criticized in its recitation of "propagation material".

In response, and with respect to claim 9, Applicants have amended this claim to amend the criticized wording. The new wording is supported by Examples 4 and 5 which are directed to anti-sense constructs based on chloroplastic GS sequences as obtained from various plant organisms, and also by page 9, last three lines, and page 10, top line which reads: "Alternatively, it may comprise an anti-sense sequence that encodes the complement of an RNA molecule or portion thereof (as described in EP 140 308 B1 and EP 223 399 B 1) in order o suppress the expression of the internally expressed glutamine synthetases."

With respect to claim 11, Applicants respectfully note that it automatically has been amended since it is dependent from claim 9. Specifically, it now recites the "imported asparagine synthetase", according to claim 9, wherein it is stated that there is a "transfer and integration of a DNA sequence coding for a prokaryotic asparagine synthetase in the plant genome". This explicitly means that no integration in any of the chloroplast or plastid genome in such a plant cell can be meant. As a result, the asparagine synthetase must be imported to the cellular parts wherein it is active, and these parts are chloroplasts and/or plastids.

With respect to the criticism of the recitation of "propagation material", in claim 12, Applicants submit that when one reviews the last paragraph at page 7 of the instant specification, there is no way that one of skill in the art would understand from the present wording "propagation material", any items such as plant cuttings, MS agar, potting soil or the like, i.e., one of skill would not understand "propagation material" to mean anything other than plant materials such as cells, seeds, and so forth, subsequent to transgenic modification. This last paragraph contains a detailed description of how to combine specific components on the basis of genes, promoters, leader sequences, so to generate gene constructs which can be used as vectors which then can be used for transforming plants. The last sentence of this paragraph then refers

back to the foregoing saying: "Thus, the invention comprehends a plant cell transformed with the gene construct or vector, as well as plants, seeds, and propagules or propagation material containing such cells". The words "such cells" thus can only mean cells which are obtained subsequent to the transformation steps that had been previously explained.

Applicants submit that the instant amendment places this application into good form, and overcomes the various rejections under 35 U.S.C. §112, second paragraph.

Claims 9 and 11 – 16 have been, or remain, rejected under 35 U.S.C. §103(a), for the reasons of record, as unpatentable over Coruzzi et al. (AG), in view of Dudits et al. (AH), Temple et al. (AB), and Della-Cioppa et al.

Applicants urge that none of the cited references, either alone, or in any fair combination, serve to teach or suggest the presently claimed invention. Applicants submit that there is a clear difference whether one intends to knock out all the present and expressed genes in a plant or if one intends to selectively knock out only one specific gene which has been identified as the relevant one, as taught by the instant invention.

In more detail, Coruzzi et al. has discussed that there may be several different Glutamine synthetases present in plants which are encoded by different genes. On page 22, lines 14-16, Coruzzi states that: "In plant species that encode multiple GS isozymes, this may require the suppression of the endogenous GS genes".

This clearly reflects the fact that in Coruzzi, "the endogenous GS genes", which equal "all GS genes", must be suppressed. Furthermore, it is well known that multigene families exist in plants in case of enzymes exhibiting an enzymatic activity of a Glutamine synthetase (GS) and it is further known that there are several kinds of GS, i.e. at least GS_1 (cytosolic) and

GS_2 (plastidic), present in plants and active in different tissues (see Temple et al, Mol, Gen. Genet. (1993), 236, 315-325).

In the present invention, it is apparent from a review of Examples 4 and 5 of the disclosure, that the only GS which was targeted is the chloroplastic GS (GS_2) because only the chloroplastic GS-coding sequence was cloned in the anti-sense orientation and not any other. Targeting only GS_2 means that all the other Glutamine synthetases should remain unaffected.

This is absolutely different from the intent to inhibit all GS genes taught by Corruzzi et al. In order to reach a high probability of inhibiting the correct and specifically selected enzyme, Applicants furthermore used a homologous gene (the gene taken from a plant such as rape seed for knocking out the respective gene in rape seed). This is described in Example 4 ("Inhibition of chloroplastic glutamine synthetase by expression of the anti-sense gene in tobacco and rape seed. The coding sequences for the chloroplastic isoenzymes of Nicotiana sylvesfris and Brassica napus were cloned by PCR methods from the genomic DNA of the respective plants". (Nicotiana sylvestras is a tobacco plant; Brassica napus is a rape seed plant)

Example 5 relates to maize and emphasizes again the fact that the anti-sense sequence must be a homologous one; as stated in Example 5 of the specification: "Inhibition of chloroplastic glutamine synthetase by expression of the respective anti-sense gene in maize. The coding sequences of the chloroplastic isoenzyme of Zea mays was cloned by PCR methods form the genomic DNA". (Zea mays is a maize plant.)

Therefore, it is submitted that the teachings of Coruzzi et al. differ totally from those of the present invention, as there is a completely different overall effect if one knocks out only a part of a set of genes (especially one - which means, in this case, the chlorplastic

Glutamine synthetase), or all genes (such as those encoding the Glutamine synthetase of the cytosol and of the chloroplasts of a given plant).

Therefore, it is submitted that it is not obvious for a skilled artisan to reduce the knockout recommendation, as taught by Corruzzi (knockout of all GS genes in a plant) to the selected knockout of only a certain species of GS genes (like the chloroplastic GS genes) as is taught by the instant present invention for two reasons: (1) The knockout recommendation in Coruzzi et al. was addressed unspecifically to any of the Glutamine synthetases present at any location in a plant cell (cytosol + chloroplasts) and (2) the employed sequence was not especially selected for being a homologous sequence (a sequence taken from an organism which should be targeted).

With respect to the remarks on page 6 of the Office Action (last 6 lines and page 7, top 4 lines), wherein it is stated that:

> "Importantly, Coruzzi teaches that where suppression of most, if not all, GS enzymes is desired, it is preferred that the co-suppression construct encodes a complete or partial copy of chloroplastic GS mRNA (e.g. pea GS2 mRNA). As disclosed herein (section 6.2.2.), such constructs are particularly effective in suppressing the expression of the target gene (p 29, lines 26-32). Section 6.2.2. discloses transgenic plants engineered to suppress chloroplast GS2 activity using antisense GS2 constructs (p.45-49); furthermore, claim 12 (p. 85) explicitly recites a method of producing a plant with a suppressed level of chloroplastic glutatnine synthetase. Taken together, when compared with the teachings of the instant specification, the teachings of Coruzzi provide an equally, if not more compelling and thoughtful case fox engineering trangenic plants expressing antisense RNAs of chloroplastic glutamine synthetase genes",

Applicants submit that it becomes clear when reviewing this statement, that Coruzzi is applying a completely different technique in order to silence certain genes, including Glutamine synthetase genes, i.e. the co-suppression technique.

Applicants further respectfully point out that in Coruzzi, at page 29, lines 26-29, it is stated that: "In embodiments, where suppression of most, if not all, GS isozymes is desired, it is preferred that the co-suppression construct encodes a complete or partial copy of chloroplastic GS mRNA."

"This "co-suppression-technology" is completely different from the "anti-sense-technology" (see Examples 4 and 5) employed by Applicants..

Applicants further urge that all constructs and observed reduction of Glutamine synthetase reduction, as described on pages 45 to 49 of Coruzzi, relate to the co-suppression approach (and not to an antisense approach - as stated by the Examiner in the Action on page 6, last two lines), as one can easily detect when reading, for example page 48, lines 19-28:

> "By contrast, the Z41 construct was less efficient at down-regulating endogenous tobacco chloroplastic GS2 and these plants showed wider range of co-suppression phenotypes (see variation in GS activity amongst Z41 individuals in Tables lA and 1B). Typically, plants co-suppressed for GS2 (/54 or Z41) grew more slowly than wild-type and developed intervenial chlorosis (see Figure 10) ...."

and page 49, lines 17-22:

> "These results suggest the specific co-suppression of tobacco ehloroplastic GS2 form the insertion of a pea GS2 transgene. In addition, the GS2 transgene was also silenced. Levels of cytosolic GS mRNA and protein were unaffected in these GS2 co-suppressed plants."

Moreover, cited Claim 12 of Coruzzi et al reads as:

> "The method of claim 11, wherein said glutamitte synthetase gene is an gene encoding chloroplaetlc glutamine synthetase."

This claim refers back to Claim 11 of Coruzzi et al., which reads:

> "A method of producing a plant with a suppressed level of glutamine synthetase by engineering the plant for ectopic overeapression of a

glutamine synthetase gene, wherein the suppressed level of glutatnine synthetase is in comparison with identically cultivated uncagineered, progenitor plant; and the engineering of the plant comprises:

i) transforming the plant with a gene fusion designed to confer ectopic overexpression of a giutamine aynthetase gene,
ii) selecting or identifying the transformed plant based on the trait conferred by a marker gene linked to said gene function,
iii) screeing the transformed plant for an abnormally low level of glutamine synthetase, and
iv) selecting the transformed plant with an abnormally low level of glutatnine synthetase"

The terms "ectopic" and "overexpression" are defined at 5.1 (page 21) of Coruzzi and can be identified without any doubt as being completely different from what is understood as an anti-sense inhibition, as these refer to co-suppression (or also named sense-suppression in some of the literature, i.e., Dougherty and Parks, Current Opinion in Cell Biology (1995), 7, 399-405, which is based on an over-expression of the gene of interest to be silenced or heavily reduced. This definition is also supported by the wording of the Coruzzi et al disclosure, page 41, lines 3-11, which reads as:

> "In some instances, however, overexpression of cytosolic GS and/or chloroplastic GS leads to a down regulation of endogenous gene expression or co-suppression. Some transformed plants containing cytosolic GS overexpression constructs and all transformed plants containing chloroplastic GS2 constructs do not overexpress GS, but rather are suppressed for GS expression, including suppression of the endogenous GS gene (i. e., co-suppression)."

When reading this, it becomes obvious that it is highly unpredictable if one will end in a down-regulation or in an up-regulation of the GS, in the respective plant; Coruzzi's approach is a shot-gun approach, rather like a lottery, and it covers all GS .

As both techniques exhibit a completely different mode of action in the transgenic plant, there is almost no correlation possible between co-suppression (or sense-suppression) (as described by Coruzzi) and anti-sense suppression (as described by Donn et al. in present invention)-which includes the different sensitivities and needs in order to specifically silence a particular gene out of a gene family. When reviewing all the other findings of Coruzzi et al., one cannot locate any claim referring to anti-sense as the matter of choice for silencing any gene; only ectopic overexpression of various genes is suggested.

Therefore, when looking to Coruzzi's disclosure, one of skill cannot compare the present invention with what has been disclosed by Coruzzi et al.; the only similarity is the fact that both are in part teachings about the regulation of Glutamine synthetase expression in plants.

Applicants submit that with respect to Temple et al., in addition to the publication of Coruzzi et al., it is apparent that - at that point in time - it was not understood in the scientific community how to control selectively the GS_1 and GS_2 genes via an anti-sense approach, as they were not able to demonstrate the selective knockout of GS_1 only in tobacco plants (see summary at page 315, left column, lines 10-15):

> "Leaves of the plants transformed with the anti-sense GS_1 construct showed a significant decrease in the level of both GS_1 and GS_2 polypeptides and activity but did not show any significant decrease in the level of endogenous GS mRNA,"

Furthermore, it is stated at page 319, right side, last paragraph:

> "Our results suggest that the antisense alfalfa GS transcript does not lower the steady-state level of the endogenous GS transcript in the heterologous plant, tobacco. Since a significant decrease in GS [and this equals GS_1, and GS_2] proteins is observed in the GS_1 antisense plants, it is likely that inhibition occurs at the level of translation".

Reading this, it is clear that one of skill will recognize the long felt need for, and significance of, the homologous anti-sense approach in case of Glutatmine synthetase, and which is a significant contribution of the instant invention. (see Examples 4 and 5).

Moreover, the following statement is given in Temple at page 322, 2nd paragraph, 1st sentence:

> "The results presented in this paper demonstrate that the full-length alfalfa GS_1 gene, when transcribed in an anti-sense orientation from the 35S promoter, is capable of downregulading both GS_1 and GS_2 in tobacco leaves. Besides demonstrating that the antisense RNA approach is effective in silencing GS gene expression, our results also show that a beterologous andaense GS_1 transcript is effective in inhibiting both GS_1 and GS_2 gene expression."

Again, this demonstrates that Temple et al. were only able or only intended to knock down all GS enzymatic activities in a transgenic plant, and not to run the knock down procedure in a highly specific manner so to address only one certain type of GS, as is taught in instant invention.

In contrast to the teachings of Temple et al., Applicants use a homologous gene in an anti-sense orientation (for example GS_2 from maize to down regulate the transcription of a GS_2 in maize (see Example 5), and not a heterologous approach as Coruzzi et al. did in the case of their transgenic plants based on the co-suppression approach, or Temple did on the heterologous approach used in case of generating transgenic plants based on an anti-sense approach; but using sequences obtained from an alfalfa cell culture for knocking down GS synthesis in tobacco plants.

Consequently, Temple et al - even in combination with Coruzzi et al. - would leave the skilled artisan with the assumption that one would not be able to run such a highly selective control of GS activities. This is contrary to what is taught by Applicants. Therefore,

the instant invention is neither taught or suggested by Temple et al., either alone or in connection with Coruzzi et al. (or any of the other references).

Again, Applicants point out that the application of at least one chemical compound, as taught by Dudits et al. in order to inhibit the GS-activity is not as selective as the GS_2-anti-sense construct taught by Applicants. This is similar to the chemical(s) described by Dudits et al., which inhibit GS_1, as well as GS_2, and therefore do not exhibit the highly specific manner of Applicants' GS_2 -antisense construct. Consequently, Dudits et al. fails to obviate the instant invention, either alone or in combination with the other references.

Furthermore, Applicants point out again that Della-Cioppa et al. discloses several possible chloroplast transit peptides. These authors report clearly that the efficiency may differ significantly from the various chimeric proteins (page 965 right column, 4th paragraph to page 966, left column, end of 2nd paragraph "Recent experiments cytosol as opposed to the absolute rate of chloroplast import"). Consequently, it is particularly important to combine an efficient transit peptide sequence with the protein of interest (which is in case in the present invention for the bacterial asparagine synthetase) to be transferred into the chloroplast. This was described in detail "Example 1" of the present invention.

Consequently there was no hint or suggestion given by Della-Cioppa et al., concerning the chimeric protein employed by Applicants which is a "modified transit peptide form the small subunit of Ribulosebisphosphat Carboxylase from pea plus a duplication of 20 amino acids compared to the natural transit peptide", page 15, lines 1-5), covalently connected to the bacterial asparagine synthetase, so that it was not expected that, for example, this combination of a transit peptide with the bacterial ASN-A obtained from E. coli would be imported into chloroplast in an efficient manner, especially in view of the fact that it is

recognized by the skilled artisan that protein folding in a living cell is a quite complex and unpredictable process, even today.

In view of the foregoing amendments and remarks, Applicants respectfully request therefore that the Section 103 rejection be reconsidered with the following in mind. First, it is well established that "there must be some reason for the combination other than the hindsight gleaned from the invention itself". Uniroyal v. Rudkin-Wiley, 5 U.S.P.Q. 2d 1434, 1438 (Fed. Cir. 1988). Second, there must be some prior art teaching which would have provided the necessary incentive or motivation for modifying the primary reference in the manner suggested by the Examiner. In re Laskowski, 12 U.S.P.Q. 2d 1397, 1399 (Fed. Cir. 1989). Third, "obvious to try" is not the standard under 35 U.S.C. §103. In re Fine, 5 U.S.P.Q. 2d 1596, 1599 (Fed. Cir. 1988). Further, as stated by the Court In re Fritch, 23 U.S.P.Q. 2d 1780, 1783-1784 (Fed. Cir. 1992):

> The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification.

As discussed above in detail, the references relied upon by the Examiner fail to provide the necessary incentive or motivation for modifying the references in a manner which would produce the invention as claimed.

In view of the foregoing, Applicants respectfully urge that the rejection does not establish a *prima facie* case of obviousness.

Moreover, even if a prima facie case of obviousness were established (although Applicants do *not* admit this for the reasons set forth above), Applicants have provided evidence of surprising and unobvious results (see instant specification, at inter alia, Example 8, "Growth behavior of transgenic maize and tobacco plants").

It is thus asserted that the claimed invention and its unexpected and surprising advantages are not taught or suggested by the cited references, either individually or in any fair combination. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are respectfully requested.

However, without waiving their position that the claims as originally presented are in patentable form, but in order to expedite prosecution and allowance, Applicants have amended their claims as set forth above, which amendments are supported by the Examples 4 and 5 and the specification on page 8 lines 1-6, and by the specification at page 15, lines 1-5. No new matter is added thereby.

Applicants submit that the instant submission renders this application into condition for allowance. Alternatively, it places this application into better condition for purposes of appeal. Applicants therefore respectfully request entry of this Amendment. As noted above, a Notice of Appeal and required fee therefor, are being filed concurrently herewith.

Please charge any additional fees required or credit any overpayment to Deposit Account No. 50-0320.

Respectfully submitted,

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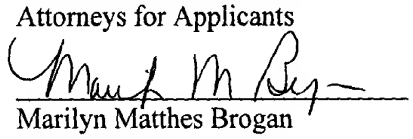
Attorneys for Applicants

By: 

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

Please rewrite the claims to read as follows:

--9. (twice amended) A process for the production of plants with improved growth characteristics, which comprises the following steps:

b) transfer and integration of a DNA sequence coding for a prokaryotic asparagine synthetase in the plant genome[.]; <u></u>

[b)] wherein said DNA sequence is linked to a regulatory sequence for the expression of said DNA sequence and import of the asparagine synthetase into the chloroplast [and/or] <u>or</u> plastids of a plant cell and wherein said plant cell exhibits the biochemical activity of the imported asparagine synthetase in its chloroplasts [and/or] <u>or</u> plastids [and]; <u></u>

[c)] <u>b)</u> transfer and integration of a [DNA sequence encoding a chloroplastic GS-antisense RNA in the plant genome] <u>chloroplastic glutamine synthetase gene or a portion threof into the plant genome which encodes and expresses an anti-sense RNA of said gene</u>

[d)] wherein the DNA sequence [under c)] is linked to a regulatory sequence for the transcription of said DNA sequence and

[e)] <u>c)</u> regeneration of intact and fertile plants from the transformed cells.

11. (twice amended) A plant cell obtainable by a method as claimed in claim 9, wherein a prokaryotic ammonium specific asparagine synthetase exhibits the biochemical

activity of the imported asparagine synthetase in its chloroplasts [and/or] or plastids and which contains a gene construct which provides a reduced level of expression of endogenous chloroplastic glutamine synthetase activity.

12. (Not further amended) A plant, seeds, propagule or propagation material containing cells according to claim 11.

13. (amended) A gene construct comprising a gene encoding a prokaryotic ammonium specific asparagine synthetase operatively linked to a regulatory sequence for the expression of said gene and import of the asparagine synthetase into the chloroplasts [and/or] or plastids of a plant cell and wherein said plant cell exhibits the biochemical activity of the imported asparagine synthetase in its chloroplasts [and/or] or plastids.

14. (amended) A gene construct according to claim 13, wherein the asparagine synthetase gene is an E. coli asparagine synthetase gene with a chloroplastic leader peptide at its N-terminus and which leader peptide is a modified transit peptide form the small subunit of the Ribulosebisphosphat carboxylase from pea containing a duplication of 20 amino acids compared to the natural transit peptide.

15. (twice amended) A vector containing a gene construct according to claim [13] 14 which gene construct comprises a sequence which encodes a chloroplastic leader peptide at its N-terminus and which leader peptide is a modified transit peptide form the small subunit of the Ribulosebisphosphat carboxylase from pea containing a duplication of 20 amino acids compared to the natural transit peptide.

16. (Not further amended) A plant cell transformed with the gene construct according to claim 13 or with vector according to claim 15. --.